ASSEMBLY AND POSITIONING OF THE OOCYTE MEIOTIC SPINDLE

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ABSTRACT

Fertilizable eggs develop from diploid precursor cells, called oocytes. Once every menstrual cycle, an oocyte matures into a fertilizable egg in the ovary. To this end, it eliminates half of its chromosomes into a small cell, called a polar body. The egg is then released into the Fallopian tube, where it can be fertilized. Upon fertilization, the egg completes the second meiotic division, and the mitotic division of the embryo starts. This review will highlight recent work that has shed light on the cytoskeletal structures that drive the meiotic divisions of the oocyte in mammals. In particular, we will focus on how mammalian oocytes assemble a microtubule spindle in the absence of centrosomes, how they position the spindle in preparation for polar body extrusion and how the spindle segregates the chromosomes. We will primarily focus on mouse oocytes as a model system, but also highlight recent insights from human oocytes. (150/150)

INTRODUCTION

When an egg is fertilized by a sperm, the chromosomes of the father and the mother are united and a new and genetically unique embryo begins to develop. The embryo will only develop correctly, if the egg and the sperm carry precisely one copy of each chromosome. However, human eggs frequently contain too many or too few chromosomes – they are aneuploid. Most aneuploidy results from chromosome segregation errors during the meiotic divisions of the egg. An egg develops from a diploid progenitor cell, called oocyte. Oocytes are stored in the ovary from birth onwards. Once every menstrual cycle, an oocyte resumes meiosis and eliminates half of its chromosomes into a small cell, called a polar body (Figure 1). The mature egg is then ovulated into the Fallopian tube, where it can be fertilized by sperm. Upon fertilization, it completes the second meiotic division and eliminates half of the remaining sister chromatids. The male and female pronuclei form and unite, and the mitotic divisions of the embryo start (Figure 1).

The cytoskeletal machineries that drive the meiotic divisions of oocytes are highly specialized. The female meiotic spindle lacks centrosomes, the main organizers of spindles in mitotic cells. Oocytes from different species have adopted different mechanisms to assemble a spindle in the absence of centrosomes. Strikingly, recent work has revealed that oocyte spindles not only require microtubules but also actin to function properly. In addition to mediating spindle assembly, centrosomes are also instrumental in positioning the spindle in many cell types: they nucleate astral microtubules which often interact with a polarized cortex and mediate asymmetric spindle positioning. Also oocytes have to position their spindle extremely asymmetrically in order to minimize the cellular

material that is eliminated together with the chromosomes during the meiotic divisions. However, oocytes lack astral microtubules and have adopted actin-based mechanisms to achieve asymmetric division. This review will summarize recent advances in our understanding of how the specialized cellular machineries are working that separate and partition chromosomes between oocyte and polar bodies during oocyte meiosis, with a focus on mechanisms that act in mammals.

1. SPINDLE ASSEMBLY IN MAMMALIAN OOCYTES

1.1 Centrosome elimination in oocytes

During mitosis, centrosomes serve as major sites of microtubule nucleation and form the two poles of the spindle (Prosser & Pelletier 2017, Vertii et al 2016). Centrosomes consist of a pair of centrioles surrounded by pericentriolar material (PCM), which contains proteins that are required to nucleate and anchor microtubules (Vertii et al 2016). Centrosomes duplicate during S-phase and are then distributed to the two daughter cells during mitosis.

However, in oocytes of several species, including mice (Szollosi et al 1972), humans (Hertig & Adams 1967), worms (Mikeladze-Dvali et al 2012), flies (Pimenta-Marques et al 2016) and others (Manandhar et al 2005), centrioles are eliminated before fertilization. In mice, centrioles are present until the pachytene stage of oogenesis (Szollosi et al 1972) and reassembled de novo in the early developing embryo (Courtois et al 2012). Centrosomes are also lost during oogenesis in *D. melanogaster* oocytes (Pimenta-Marques et al 2016). Here, elimination occurs in a stepwise manner: first, pericentriolar material components are lost, followed by the disappearance of centriolar proteins in the late stages of oogenesis before meiotic spindle assembly. In contrast,

starfish oocytes eliminate their centrosomes during the meiotic divisions: in meiosis I, two centrosomes, each containing a pair of centrioles, form the spindle poles. During anaphase I, one of these centrosomes is eliminated into the first polar body. The other one remains in the egg, and splits into two centrosomes with only one centriole each, which then form the metaphase II spindle. During anaphase II, the mother centriole is eliminated into the second polar body and the remaining daughter centriole is degraded in the egg's cytoplasm (Sluder et al 1989). A recent study of starfish eggs has revealed how the mother centriole is selectively eliminated (Borrego-Pinto et al 2016). During meiosis II, the spindle pole with the mother centriole is positioned close to the cell membrane. Spindle positioning depends on the minus-end directed motor dynein, but the precise mechanism is still unclear. The mother centriole is then anchored to the cell membrane via its appendages and subsequently eliminated into the second polar body (Borrego-Pinto et al 2016). The remaining daughter centriole is then inactivated, as judged by loss of microtubule nucleation activity, and ultimately degraded by unknown mechanisms.

Although it is still unclear why oocytes lack centrosomes, several possible explanations have been proposed (Manandhar et al 2005). In organisms where the sperm introduces a centrosome, centrosomes may need to be eliminated in the egg to avoid that the zygote has too many centrosomes (Manandhar et al 2005). Lack of centrosome-nucleated astral microtubules that push the spindle away from the cortex may also promote extrusion of very small polar bodies: the closer the spindle is to the cortex, the smaller the amount of cytoplasm that is eliminated into the polar body (discussed by (Severson et al 2016). Also the lack of an S-phase between the two meiotic divisions, in

which centrosomes are normally duplicated, results in problems with centriole numbers, as exemplified by meiosis in starfish oocytes.

1.2 Acentriolar MTOCs replace centrosome function in oocytes of many species

Instead of centrosomes, many oocytes have distinct microtubule organizing centers (MTOCs) that lack centrioles. For instance, frog oocytes have a large flat MTOC near the nucleus that drives spindle assembly (Gard 1992); and in worm oocytes, several MTOCs coalesce to form the spindle poles (Connolly et al 2015). In *Drosophila* oocytes, MTOCs may also promote meiotic spindle assembly (Skold et al 2005). Acentriolar MTOCs (aMTOCs) are also present in mouse oocytes and contain several centrosome proteins including pericentrin (Carabatsos et al 2000), γ-tubulin (Gueth-Hallonet et al 1993) and its partner NEDD1 (Ma et al 2010), and Cep192 (Clift & Schuh 2015). aMTOCs serve as a major site of microtubule nucleation and functionally replace centrosomes in mouse oocytes (Schuh & Ellenberg 2007). Whether aMTOCs participate in meiotic spindle assembly in human oocytes is unclear (Battaglia et al 1996a, Battaglia et al 1996b, George et al 1996, Holubcova et al 2015, Pickering et al 1988). Instead, chromosome-dependent nucleation of microtubules plays a major role in human oocyte spindle assembly (Holubcova et al 2015).

1.3 Chromatin-dependent microtubule nucleation

That chromosomes can drive spindle assembly was first discovered over two decades ago. The introduction of DNA-coated beads into frog egg extracts that lack centrosomes led to self-assembly of a spindle structure around the DNA (Heald et al 1996). This seminal study demonstrated that chromosomes were no longer mere passengers of the spindle machinery, but could help to build it.

Chromatin-dependent mechanisms of microtubule nucleation and spindle assembly have since been studied in great detail. The chromosomes promote spindle assembly by local activation of the small GTPase Ran (Carazo-Salas et al 1999). Ran in turn releases microtubule nucleation factors, motors proteins and other microtubule-associated proteins from inhibitory binding to importins, promoting the assembly of microtubules in proximity of the chromosomes (Clarke & Zhang 2008, Petry 2016).

Ran-mediated microtubule nucleation pathways are of major importance for spindle assembly in frog egg extracts and cell-free systems (Halpin et al 2011, Kalab et al 2006). They also facilitate the rapid assembly of the spindle in mitotic cells, but are not essential to form a mitotic spindle (Maresca et al 2009). Also in mouse and frog oocytes, inhibition of the Ran-GTP pathway does not completely block spindle assembly (Bury et al 2017, Dumont et al 2007b, Schuh & Ellenberg 2007). aMTOC-driven spindle assembly is dominant over the Ran-GTP pathway in mouse oocytes: spindles that eventually form in the absence of the Ran-GTP pathway fail to assemble when aMTOCs are also disrupted (Baumann et al 2017). In contrast, Ran-GTP-mediated nucleation of microtubules from the chromosomes is of major importance for spindle assembly in human oocytes, suggesting that it is particularly important in oocytes that lack prominent MTOCs (Holubcova et al 2015).

1.4 Spindle assembly in mouse oocytes

The mechanism by which aMTOCs mediate spindle assembly is best understood in mice. Participation of aMTOCs in meiotic spindle assembly was suggested in early immunofluorescence studies of mouse oocytes (Maro et al 1985, Van Blerkom 1991) and later confirmed by live cell microscopy (Schuh & Ellenberg 2007). Upon release from

prophase arrest, multiple aMTOCs start to nucleate microtubules throughout the cytoplasm and on the nuclear envelope. They then converge onto the oocyte nucleus where they nucleate microtubules that grow at similar speeds as mitotic microtubules (Figure 2) (Schuh & Ellenberg 2007). On the nuclear envelope, aMTOCs are exposed to extensive stretching forces and some degree of fragmentation via the action of microtubules and the minus end-directed motor dynein: depolymerization of microtubules, blocking dynein's activity or detaching dynein from the nuclear envelope by removal of its adapter BicD2 all prevent aMTOC stretching (Figure 2) (Clift & Schuh 2015, Luksza et al 2013). After nuclear envelope breakdown (NEBD), aMTOCs are further fragmented and redistributed into many smaller aMTOCs, this time driven by the kinesin-5 family member KIF11 (Clift & Schuh 2015). Soon after NEBD, a Ran-dependent surge in the nucleation activity of fragmented aMTOCs generates a microtubule ball onto which chromosomes are distributed in a circular fashion (Schuh & Ellenberg 2007).

The aMTOCs are then expelled outward to the surface of the microtubule ball by the antiparallel microtubule-sliding motor activity of KIF11 (Figure 2) (Schuh & Ellenberg 2007). Gradual clustering of multiple aMTOC poles into two dominant poles then transforms this multipolar intermediate into a bipolar structure. Sorting of aMTOCs into spindle poles involves hepatoma up-regulated protein (HURP) (Breuer et al 2010), a microtubule-associated protein that stabilizes microtubules in the vicinity of mitotic chromosomes (Koffa et al 2006, Sillje et al 2006). Its depletion in mouse oocytes lowers the density of microtubules during spindle assembly and leads to failure in aMTOC sorting and spindle bipolarization (Breuer et al 2010). Similarly to mitosis, nuclear mitotic apparatus (NuMA) (Merdes et al 1996) stabilizes acentriolar spindle poles by anchoring

microtubule minus ends in mouse oocytes (Kolano et al 2012). Merging smaller aMTOC foci into poles could be an evolutionarily conserved pathway of building spindle poles because coalescence of foci is also apparent in oocytes of worms and flies (Connolly et al 2015, Skold et al 2005). Mechanistic details of aMTOC clustering in these and other model systems were discussed in recent reviews (Bennabi et al 2016, Severson et al 2016).

1.5 Spindle assembly in human oocytes

Chromosome segregation errors in human oocytes frequently result in the fertilization of aneuploid eggs. The reasons for why a process so vital to human life is prone to mistakes has been the topic of extensive research spanning decades. However, we are only just beginning to understand the mechanisms of chromosome segregation during female meiosis in humans. Well-studied causes of chromosome mis-segregation such as the age-dependent loss of cohesion have been covered in recent reviews (Capalbo et al 2017, Herbert et al 2015, Webster & Schuh 2017). Here, we will limit our discussion to highlight recent findings that have advanced our understanding of chromosome segregation mechanisms in human oocytes.

Advances in the field of meiosis research in human oocytes were hindered by the limited availability of cells for use in research, compounded by the lack of microscopy assays for studying meiotic chromosome segregation in living human oocytes. Collaboration with fertility clinics and innovation of the necessary live imaging techniques has recently provided new insights into how meiosis progresses and how the spindle is assembled in human oocytes (Holubcova et al 2015). Human oocytes assemble a spindle in a lengthy process that does not involve prominent aMTOCs (Holubcova et al 2015).

Upon nuclear envelope breakdown, the oocytes first cluster their chromosomes into an aggregate. Only several hours later, microtubules start to be nucleated from within the aggregate and the chromosome's kinetochores. The microtubule mass then slowly but continuously increases over several hours. This increase in microtubule nucleation is strictly dependent on the small GTPase Ran, and therefore mainly chromosome-driven. As the spindle assembles, it undergoes extensive reorganization. During this reorganization phase, the spindle frequently fragments into multipolar spindle intermediates, which then merge again into a bipolar spindle structure. As the spindle reorganizes, the chromosomes become aligned in the spindle center in a stepwise manner. However, chromosomes frequently remain incorrectly attached to the spindle poles. During meiosis I, the two sister kinetochores should be oriented and linked towards the same spindle pole. But in human oocytes, they are often attached to opposite spindle poles. These merotelic attachments are a frequent cause of lagging chromosomes during anaphase in cancer cells, and have been suggested to be favoured by multipolar spindle intermediates (Ganem et al 2009). Multipolar spindle intermediates also correlate with lagging chromosomes in human oocytes, suggesting that spindle instability may lead to increased baseline aneuploidy in this cells type. The spindles that are formed before anaphase onset in human oocytes also have a rather special morphology: they consist of loosely clustered short microtubule bundles and have very broad spindle poles. Further investigation is required to identify additional molecular players that are involved in their formation.

2. CHROMOSOME SEGREGATION IN MAMMALIAN OOCYTES

Chromosome segregation errors are a leading cause of aneuploidy in eggs (Hassold & Hunt 2001). The frequency of aneuploid eggs increases with age, a correlation often referred to as the 'maternal age effect' (Herbert et al 2015, Nagaoka et al 2012). To segregate chromosomes equally between the oocyte and the polar body, the chromosomes are first aligned on the spindle equator. During anaphase, shortening of a stable population of microtubules that are connected to the kinetochores (k-fibers) pulls the chromosomes towards opposite spindle poles (Cheeseman & Desai 2008, Maiato et al 2004).

2.1 Chromosome alignment and segregation in mouse oocytes

Meticulous tracking of kinetochores and chromosomes in live mouse oocytes has revealed that chromosome alignment on the meiosis I spindle is established in distinct phases (Kitajima et al 2011, Schuh & Ellenberg 2007). First, kinetochores and chromosomes are individualized on the surface of the microtubule ball. Next, the ball extends and the chromosomes form a loose ring around the forming ellipse, called 'prometaphase belt' (Figure 2). The chromosomes then invade the spindle and form the metaphase plate (Kitajima et al 2011).

In meiosis, recombined homologous chromosome pairs are held together and arranged into a meiosis-specific bivalent structure (Figure 1). During meiosis I, the two sister kinetochores of each chromosome are paired and face towards the same spindle pole. In mouse oocytes, the pairing of sister kinetochores is dependent on the meiosis-specific kinetochore protein MEIKIN (Kim et al 2015). Chromosomes are bioriented only after completion of chromosome congression and during elongation of the microtubule

ball into a bipolar spindle (Kitajima et al 2011). However, most kinetochore-microtubule interactions are initially incorrect and go through multiple rounds of correction before they are eventually stabilized (Kitajima et al 2011). This intrinsically error-prone process becomes even more defective with increasing age in mice (Shomper et al 2014), suggesting that incorrect kinetochore-microtubule interactions may contribute to age-related aneuploidy in eggs, independently of cohesion loss (Herbert et al 2015). This problem may be further exacerbated by recently reported microtubule dynamics defects in aged oocytes (Nakagawa & FitzHarris 2017). Analysis of microtubules in spindles from young and aged mouse oocytes revealed differences in microtubule growth rates and organization. In particular, older oocytes contained fewer stable microtubules associated with kinetochores. Importantly, changes in microtubule dynamics are intrinsically coupled to advanced maternal age: substituting old chromosomes for young chromosomes by nuclear transfer did not prevent the formation of defective multipolar spindles in aged oocytes (Nakagawa & FitzHarris 2017).

Once chromosomes are fully bioriented on the metaphase plate, they are partitioned towards the opposite spindle poles. In mitotic cells, shortening of k-fibers (anaphase A) first separates the chromosomes, which are then pulled apart even further by spindle elongation (anaphase B). Surprisingly, in mouse oocytes anaphase B spindle elongation precedes anaphase A (FitzHarris 2012). Early onset of anaphase B is driven by the kinesin motor KIF11 and is coupled to loss of kinetochore-microtubule attachments during anaphase A (FitzHarris 2012).

2.2 Spindle actin promotes accurate chromosome segregation

Recent work has identified an actin-dependent mechanism that promotes the accurate segregation of chromosomes in oocytes. Actin is generally thought to be dispensable for chromosome segregation. However, the microtubule spindle in mouse oocytes (Azoury et al 2008, Schuh & Ellenberg 2008) as well as human, pig and sheep oocytes (Mogessie & Schuh 2017) is permeated by prominent actin filaments (spindle actin), whose function was unknown. Surprisingly, we found that the accurate segregation of chromosomes in both meiotic divisions of mouse oocytes critically relies on actin (Figure 3). Pharmacological or genetic disruption of actin leads to lagging chromosomes during anaphase I and II (Mogessie & Schuh 2017), independently of previously reported functions of actin in vesicle transport (Schuh 2011), spindle positioning (Azoury et al 2008, Schuh & Ellenberg 2008) and cytokinesis (Kubiak et al 1991). Disruption of actin throughout maturation or acutely in meiosis II also leads to chromosome misalignment in eggs. These alignment defects directly lead to aneuploidy, because eggs progress into anaphase II with misaligned chromosomes (Mogessie & Schuh 2017).

Loss of actin significantly reduced the density of k-fibers, whereas enriching spindles in actin increased k-fiber density. These data suggest that actin promotes the formation of k-fibers (Figure 3) (Mogessie & Schuh 2017). The shortening of k-fibers drives the segregation of chromosomes during anaphase (Cheeseman & Desai 2008, Maiato et al 2004). The observation that chromosomes frequently lag when actin is depolymerized in oocytes therefore supports our model of actin-dependent k-fiber formation (Figure 3).

The surprising involvement of actin in the formation of kinetochore fibers may also be conserved beyond mammals. Notably, actin has been reported in meiotic spindles of a variety of species including spermatocytes of insects (Silverman-Gavrila & Forer 2000), maize sporocytes (Staiger & Cande 1991) as well as frog oocytes (Weber et al 2004). Moreover, there is evidence that myosins and actin are required for accurate spindle assembly during meiosis in several species (Sandquist et al 2011). For instance, myosin 10 is required for spindle assembly in frog oocytes (Weber et al 2004). Furthermore, acute disruption of actin filaments during anaphase in insect spermatocytes has been reported to influence the movement of chromosomes (Forer & Pickett-Heaps 1998). In starfish oocytes, where cytoplasmic microtubules are too short to capture the kinetochores, a contractile network of actin transports the chromosomes within reaching distance of microtubules (Lenart et al 2005). That spindle actin is also present in human, pig, sheep and possibly other mammalian oocytes (Mogessie & Schuh 2017) hints at a conserved function of actin in safeguarding chromosome segregation during meiosis.

2.3 Chromosome alignment and segregation in human oocytes

In human oocytes, not only spindle instability but also the unique configuration of the bivalent chromosomes represents a challenge for chromosome segregation. Unlike in mitosis, sister kinetochores in meiosis I are connected to microtubules emanating from the same spindle pole, which allows the segregation of homologous chromosomes but not sister chromatids during anaphase I (Figure 4). The kinetochores of sister chromatids that make up each homolog (sister kinetochores) are fused in many species (Corbett et al 2010, Kim et al 2015, Li & Dawe 2009, Sarangapani et al 2014). In comparison, instead of being paired into one functional unit, the sister kinetochores of human bivalent

chromosomes are split and interact with microtubules as two separate units, already in oocytes from young women (Zielinska et al 2015, Patel et al 2015). The degree of kinetochore separation increases further as women get older (Zielinska et al 2015, Patel et al 2015). Split kinetochores are more frequently merotellically attached than fused kinetochores (Zielinska et al 2015). They also allow bivalents to rotate on the spindle, and to orient as in mitosis, instead of orienting like in meiosis, with both sister kinetochores facing towards opposite poles (inverted bivalents) (Figure 4) (Zielinska et al 2015). Bivalents also frequently contain large gaps between their chromosome arms and tend to separate into univalents (premature separation of sister chromatids) before anaphase onset (Sakakibara et al 2015, Zielinska et al 2015). These univalents typically align on the spindle and behave like mitotic chromosomes. Both univalents as well as inverted bivalents become more frequent as women get older, suggesting that they are a major factor contributing to the increase in aneuploid eggs with advanced maternal age. Indeed, a recent genetic study has reported that “reverse segregation patterns”, a pattern in which chromosomes behave like mitotic chromosomes in meiosis I, are a frequent contributing factor to aneuploidy in human eggs (Ottolini et al 2015).

3. ASYMMETRIC OOCYTE DIVISION

Chromosome segregation in oocytes involves two highly asymmetric divisions, in which half of the homologous chromosomes (meiosis I) and half of the sister chromatids (meiosis II) are eliminated into two small cells called polar bodies. Recent work has shed light on how these extremely asymmetric divisions are achieved.

3.1 Biological significance of asymmetric oocyte division

Despite dividing twice, the oocyte needs to preserve as much cytoplasm as possible as possible, as the cytoplasm contains energy resources, maternal mRNAs and proteins that are required for embryo development (Brunet & Verlhac 2011, Li et al 2010). To this end, it eliminates the surplus chromosomes into two small polar bodies, which contain only little cytoplasm and are often degenerated before fertilization (Schmerler & Wessel 2011).

Spindle positioning in many mitotic cells crucially relies on the microtubule cytoskeleton (Kotak & Gonczy 2013). The spindle's centrosomes nucleate astral microtubules which interact which interact with the cell cortex in a dynein-dependent manner and help to position the spindle. Polarization of the cortex results in an asymmetry of forces and is typically involved in positioning the spindle asymmetrically.

In contrast, meiotic spindles in oocytes lack centrosomes at their poles and are devoid of prominent astral microtubules (Sathananthan et al 2006, Schuh & Ellenberg 2007, Szollosi et al 1972). Thus, oocytes utilize specialized strategies to position the spindle asymmetrically. Amongst mammals, the mechanisms underlying asymmetric oocyte division is best understood in the mouse, which will be the focus of this review.

3.2 Actin and microtubule networks in mouse oocytes

Mouse oocytes undergo several transitions between symmetric and asymmetric organization as they progress through meiosis and become fertilized (Figure 1). During early oocyte development, the nucleus is often off-centered. As the oocyte grows in the follicle and acquires the competence to resume meiosis, the nucleus becomes centralized (Brunet & Maro 2007). Subsequently, the meiosis I spindle assembles at the center and migrates to the oocyte surface where the first polar body will be extruded. Afterwards, the

second meiotic spindle forms beneath the plasma membrane and is anchored at this asymmetric position during metaphase II arrest. Upon fertilization, the pronuclei form at the periphery of the zygote and migrate towards its center. Pronuclear migration and the centration of the spindle in the zygote ensure that the first mitotic division is symmetric, and that both blastomeres obtain equal amounts of cytoplasm (Chaigne et al 2016).

The actin and microtubule cytoskeleton have crucial roles in establishing and breaking symmetry in oocytes. During prophase I arrest, an interphase-type network of microtubules is present in the cytoplasm (Luksza et al 2013). In addition, a dense actin meshwork organized from linear interconnected filaments fills the cytoplasm that surrounds the nucleus (Azoury et al 2008, Schuh 2011, Schuh & Ellenberg 2008). This cytoplasmic actin network is generated by the actin nucleator Formin-2 (Azoury et al 2008, Dumont et al 2007a, Schuh & Ellenberg 2008) in cooperation with the spire-type actin nucleation factors Spire1/2 (Pfender et al 2011). It was revealed that Formin-2 and Spire1/2 colocalize with Rab11a to nucleate actin filaments from the surface of vesicles in mouse oocytes (Schuh 2011). Blocking Rab11a-positive vesicle formation by a dominant-negative variant released actin nucleators into the cytoplasm, and increased the network density (Holubcova et al 2013), consistent with a model in which sequestration of actin nucleation factors at vesicles and at the plasma membrane helps to control the network density.

In mouse oocytes, vesicles are connected with each other and the cell surface through actin filaments. This actin-vesicle network has been implicated in long-range transport towards the plasma membrane (Schuh 2011). Vesicles move towards

neighboring vesicles and towards the cell surface along cytoplasmic and cortical actin filaments in a Myosin-5b-dependent manner, generating an outward-directed motion.

3.3 Nuclear positioning in mouse oocytes

Actin may be a key player in the process that leads to nuclear centering in growing oocytes. In *Fmn2*$^{-/-}$ oocytes where the cytoplasmic actin network is absent, the nucleus is often off-centered (Azoury et al 2011, Azoury et al 2008, Dumont et al 2007a, Schuh & Ellenberg 2008). Restoring the actin network in *Fmn2*$^{-/-}$ oocytes by microinjection of Formin-2 mRNA drives the nucleus to the oocyte center (Figure 5) (Almonacid et al 2015). Nuclear centering also depends on Myosin-5b-driven vesicle motion: blocking Myosin-5b activity using its dominant-negative variant blocks nuclear centering. Furthermore, it was shown that vesicles move at faster speeds when near the oocyte surface (Figure 5) (Almonacid et al 2015). Mathematical modeling suggests that such differences in vesicle motility may create a pressure gradient and propulsion forces that could help to center the nucleus. This mechanism may also be conserved in zygotes because inhibition of Myosin-5b blocks centering of the female and male pronuclei (Chaigne et al 2016).

In contrast, maintenance of the nucleus at the center may depend on microtubules, as nuclei become off-centered when microtubules but not actin filaments are depolymerized (Figure 5) (Alexandre et al 1989). This off-centering is suppressed, when both microtubules and actin are simultaneously depolymerized. This suggests that microtubules might help to maintain the oocyte nucleus in a central position by counteracting actin-dependent forces that would otherwise drive the nucleus towards the periphery.

3.4 Spindle migration during meiosis I in mouse oocytes

Upon resumption of meiosis I in mouse oocytes, the nucleus breaks down and the spindle assembles at the oocyte center. 3-5 hours after nuclear envelope breakdown (NEBD), it relocates to the cortex where the chromosomes are segregated. Despite the nuclear centration mechanisms discussed earlier, the nucleus in mouse oocytes is still slightly off-centered before NEBD (Schuh & Ellenberg 2008). Consequently, the spindle assembles with one of the poles closer to the oocyte surface. The spindle does not migrate to a predetermined site, but always along its long axis in the direction of the pole that is closer to the oocyte surface (Schuh & Ellenberg 2008, Verlhac et al 2000).

In contrast to mitosis, spindle relocation in meiosis is independent of microtubules and solely relies on actin: depolymerization of actin but not microtubules perturbs spindle migration (Longo & Chen 1985, Schuh & Ellenberg 2008, Verlhac et al 2000).

Several actin structures have been implicated in asymmetric spindle positioning. One of these structures is the cytoplasmic actin network (Azoury et al 2008, Schuh & Ellenberg 2008). Asymmetric spindle positioning fails in oocytes that lack the cytoplasmic actin network, due to the absence of Formin-2 or Spire1/2 (Dumont et al 2007a, Pfender et al 2011). Also the transport of vesicles along the network is required for asymmetric spindle positioning, as asymmetric spindle positioning fails when Myosin-5b or Rab11a are inhibited (Holubcova et al 2013).

Previous observations of the actin network in live oocytes suggested that actin filaments are pulled into the spindle and enriched at spindle poles (Azoury et al 2008, Schuh & Ellenberg 2008). Consistently, spindle pole-generated forces pull the oocyte cortex inwards. These forces may depend on Myosin-2, another actin-based motor. The

active form of Myosin-2 localizes to the spindle poles. Blocking Myosin-2 function by drug-mediated inhibition of the Myosin light chain kinase or by antibody injection slows down spindle relocation and stops pole-directed motion of actin filaments (Schuh & Ellenberg 2008, Simerly et al 1998). Based on these findings, a model was proposed where connection of the meiotic spindle to the vesicle-actin-network supports its migration to the cortex. In this model, Myosin-2 links the spindle to the cytoplasmic actin network, and mediates the outward-directed movement of the spindle in concert with Myosin-5b, which drives the outward-directed motion of vesicles and their associated actin filaments, which are linked to the spindle (Figure 6) (Holubcova et al 2013, Schuh & Ellenberg 2008).

In addition to the cytoplasmic meshwork, the actin cortex, a thick actin layer that surrounds the oocyte surface, also plays a crucial role in asymmetric spindle positioning (Chaigne et al 2013). After NEBD, the actin cortex becomes thicker and an inner layer of actin (the subcortex) forms underneath the cortex. Formation of the subcortex and cortical thickening requires another actin nucleator, the Arp2/3 complex (Azoury et al 2008). Cortical thickening, which is coincidental with Myosin-2 removal from the cortex (Chaigne et al 2015), is proposed to soften the cortex and to thereby facilitate spindle relocation (Chaigne et al 2013). Oocytes in which the Arp2/3 complex is inhibited by drug treatment have a thinner cortex, and often divide symmetrically. Interfering with cortical plasticity by artificially stiffening or softening the cortex leads to symmetric divisions (Chaigne et al 2013). This suggests that, to support spindle positioning, cortical softness needs to be fine-tuned (Chaigne et al 2015).

3.5 Spindle positioning during meiosis II in mouse oocytes

In meiosis II, the spindle is anchored and oriented parallel to the egg's surface. It rotates into a radial position upon activation or fertilization before extrusion of the second polar body (Liu et al 2000, Maro et al 1984). Spindle anchorage is controlled by the Arp2/3 complex, a nucleator of branched actin filaments (Figure 6) - drug-mediated inhibition of Arp2/3 leads to spindle detachment from the cortex (Yi et al 2011). Rotation of the spindle also relies on an intact actin cytoskeleton (Maro et al 1984, Zhu et al 2003) but the precise underlying mechanisms are still largely unclear.

The activity of the Arp2/3 complex is dependent on nucleation-promoting factors, whose activation in turn depends on upstream signals, such as small GTPases (Rotty et al 2013). Likewise in meiosis, local recruitment and activation of the Arp2/3 complex in the actin cap, an actin-enriched zone in the cortex, is induced by chromosomes in a nucleation-promoting factor- and Ran-dependent manner (discussed later) (Sun et al 2011, Yi et al 2013, Yi et al 2011). Specifically, a dominant-negative variant of the nucleation-promoting factor N-WASP (neuronal Wiskott-Aldrich syndrome protein) cause the spindle to detach from the cortex in meiosis II, demonstrating that Arp2/3 activity at the actin cap can be regulated by N-WASP (Yi et al 2011). However, this observation could not be reproduced in oocytes obtained from *N-WASP*$^{-/-}$ mice (Wang et al, 2016), possibly due to redundant mechanisms of promoting actin nucleation by Arp2/3. For example, WAVE2 (Wiskott-Aldrich syndrome protein family member 2), another nucleation-promoting factor, has also been shown to control Arp2/3 activation at the cortex during meiosis I (Chaigne et al 2013). Thus, the exact molecular details of Arp2/3 regulation in spindle anchorage remain elusive.

Arp2/3 and nucleation-promoting factors such as N-WASP localize to the actin cap and nucleate branched actin filaments (Figure 6). This is proposed to generate an actin flow that streams away from the cortical cap along the cell periphery and converges at the opposite side of the cell before circulating back towards the oocyte center. This actin flow has been suggested to translate into cytoplasmic streaming that pushes the spindle towards the cortex (Figure 6) (Yi et al 2013, Yi et al 2011). Upon Arp2/3 inhibition, the direction of cytoplasmic streaming is reversed and pushes the spindle away from the cortex (Yi et al 2011). When Arp2/3 and Myosin-2 function are simultaneously blocked, cytoplasmic streaming is abolished and the spindle remains at cortex (Yi et al 2011). Consistently, spindle anchorage is not affected by depolymerization of actin with the drug Latrunculin A, which renders Arp2/3 and Myosin-2 inactive. This suggests that Arp2/3 may maintain the meiotic spindle at the cortex by counteracting or preventing Myosin-2-driven contractions that push the spindle towards the oocyte center.

3.6 Cortical polarization during asymmetric division

Asymmetric spindle positioning in many cell types depends on a polarized cortex, which interacts differentially with astral microtubules that are emanating from the two spindle poles (Cowan & Hyman 2004). Cortical polarity also plays an important role during meiosis in many animal oocytes. For example, in starfish and frogs, the oocyte nucleus is positioned at the future animal pole through pulling forces exerted by microtubules that extend from the cortex (Gard 1993, Miyazaki et al 2000, Miyazaki et al 2005). In contrast, in flies, MTOCs are concentrated at the posterior cortex and push the nucleus towards the anterior pole (Zhao et al 2012).

On the other hand, mammalian oocytes are generally thought to be unpolarized before meiosis resumes, and the polarity of the egg is not thought to determine the axis of the embryo (reviewed in (Johnson 2009)).

3.6.1 Chromosome-mediated formation of the actin cap

The first sign of cortical polarization in mouse oocytes is the appearance of the actin cap, which forms as the spindle moves towards the oocyte cortex, and is conserved from invertebrates to mammals (Gard et al 1995, Kim et al 2000, Sardet et al 1992, Sun et al 2001). Cortical polarization in mouse oocytes is likely to serve several different functions, including preparation of the cell surface for polar body extrusion, anchorage of the metaphase II spindle and prevention of sperm binding in the region of the metaphase II spindle. The contribution of cortical polarization to polar body extrusion is not subject of this review and is summarized elsewhere (Maddox et al 2012).

The formation of the actin cap coincides with spindle migration: when the chromosomes come closer to the cortex during meiosis I, actin becomes enriched in the cortical region that is proximal to the chromosomes (Deng et al 2007, Longo & Chen 1985, Yi et al 2013). Cortical polarization is mediated by chromosomes, because DNA-coated beads are sufficient to induce actin cap formation (Deng et al 2007). The small GTPase Ran concentrates and forms a gradient around meiotic chromosomes (Dumont et al 2007b) and is required to form the actin cap (Figure 6) (Deng et al 2007). Ran-GTP mediates the formation of the actin cap by driving the local recruitment and activation of Arp2/3 via N-WASP (Yi et al 2011). The actin cap is therefore maintained by a positive feedback loop: the chromosomes induce cortical polarization, which in turn will push the chromosomes closer to the cortex and help to maintain the actin cap (Yi et al 2013, Yi et

al 2011). The dependence of the actin cap on Ran-GTP may also explain why metaphase II spindles detach from the cortex when Ran is inhibited (Yi et al 2013).

Actin cap formation and spindle anchorage during meiosis II do not only depend on Ran. For example, Arp2/3 activity is also controlled by the Mos-MAPK pathway via the nucleation-promoting factor WAVE2 (Chaigne et al 2013, Deng et al 2007). Blocking MAPK function leads to disappearance of the actin cap above peripheral chromosomes (Deng et al 2005). Consistently, DNA beads fail to induce the actin cap in *Mos*$^{-/-}$ oocytes (Deng et al 2007). Thus, Mos-MAPK and Ran pathways work in parallel to promote Arp2/3-mediated formation of the actin cap.

Furthermore, as visualized by fluorescent probes of active GTPases, active Cdc42 and Rac, members of the Rho family of GTPases, accumulate at the actin cap in a Ran-GTP-dependent manner (Dehapiot et al 2013, Halet & Carroll 2007). Similarly to Ran, dominant-negative Cdc42 inhibition results in loss of the actin cap, probably due to N-WASP delocalization and consequently Arp2/3 deactivation (Dehapiot et al 2013, Yi et al 2011). In agreement, *Cdc42*$^{-/-}$ mouse oocytes lack cortical polarization despite normal spindle migration in meiosis I (Wang et al 2013). Thus, Cdc42 is a downstream effector of Ran and functions upstream of Arp2/3 activation.

It was suggested that Cdc42 might also contribute to spindle positioning during meiosis I: its inhibition by a dominant-negative variant led to failure in spindle migration (Na & Zernicka-Goetz 2006). However, this could not be confirmed in a subsequent study, where spindle migration was unaffected in *Cdc42*$^{-/-}$ oocytes (Wang et al 2013). This discrepancy may be explained by the use of different techniques in these studies for interfering with Cdc42 function. The effect of Cdc42 depletion in *Cdc42*$^{-/-}$ oocytes may

have been masked by redundant pathways. Further methods of acute protein inhibition or depletion are needed to investigate a potential involvement of Cdc42 in spindle migration.

Although it is required for spindle anchoring, the actin cap alone is not sufficient to maintain the spindle at the cortex: dominant-negative Rac inhibition detaches the meiosis II spindle from the cortex without disrupting the actin cap (Halet & Carroll 2007). Importantly, cortical Myosin-2 is lost in cultured cells expressing constitutively active Rac (van Leeuwen et al 1999). Based on these data, it can be speculated that Rac activity helps to displace cortical Myosin-2, which is proposed to counteract Arp2/3-driven cytoplasmic streaming (Yi et al 2011).

3.6.2 Cortical polarization prevents premature sperm-egg DNA interactions

Unpolarized mouse oocytes are covered homogenously with membrane microvilli that promote sperm-egg binding and fusion (Runge et al 2007). Cortical polarization is accompanied by loss of membrane microvilli at the actin cap (Longo & Chen 1985). This probably prevents sperm entry in the vicinity of the oocyte spindle, which is necessary to avoid that sperm DNA is captured by spindle microtubules and extruded into the second polar body (Luo et al 2009).

In mouse oocytes, proteins of the ERM family (Ezrin-Radixin-Moesin) are required for assembly and maintenance of microvilli (Yonemura et al 1999). Active forms of ERM proteins localize to the oocyte cortex but are gradually excluded from the region above the approaching spindle, thereby creating a microvilli-free zone (Dehapiot & Halet 2013, Larson et al 2010). A homogeneous distribution of active ERM at the cortex is observed after Ran inhibition (Dehapiot & Halet 2013). This indicates that Ran-GTP-mediated

polarization is required for the disassembly of microvilli at the actin cap, which is essential to prevent sperm entry near the oocyte spindle.

The mechanisms that prevent a clash between the sperm and the spindle of the egg have been characterized particularly well in *C. elegans*. In this system, the oocyte must not only prevent sperm entry in vicinity of the oocyte's spindle apparatus, but also avoid that the oocyte spindle is capture by the sperm associated microtubule aster (McNally et al 2012, McNally et al 2010). To achieve this, Kinesin-1 relocates the oocyte nucleus away from the future sperm entry site prior to NEBD. In addition, Kinesin-1 prevents premature assembly of centrosomes from sperm centrioles and astral microtubule nucleation after fertilization. While relocation probably depends on Kinesin-1's motor activity (McNally et al 2010), prevention of premature centrosome assembly relies on a Kinesin-1 shell that forms around the sperm centrioles and blocks recruitment of maternal pericentriolar proteins (McNally et al 2012). Interestingly, also actin has now been found to tether sperm DNA at the posterior pole, away from the oocyte spindle (Panzica et al 2017). Here, cortical actin directly associates with sperm components to shield sperm chromosomes. This interaction prevents the premature capture of oocyte chromosomes by sperm aster microtubules.

Sperm asters have also been detected in zygotes of some mammals including humans (Schatten & Sun 2011), raising the question whether similar mechanisms exist in these species that prevent the premature interaction between maternal and paternal structures. Interestingly, actin plays a converse role in mouse zygotes where it promotes the unification of the male and female pronucleus in the center of the cell (Chaigne et al 2016). The actin-dependent centration of pronuclei has also been proposed to rely on the

vesicle dynamics that position the oocyte nucleus and the spindle in meiosis I (Figures 5 and 6). Thus, actin-dependent mechanisms play multiple crucial roles at the transitions between symmetric and asymmetric divisions in mouse oocytes and zygotes.

PERSPECTIVE

Recent years have seen significant conceptual advances in the field of female meiosis research. Advanced microscopy technologies have allowed identification of aMTOCs as major drivers of spindle assembly in mouse oocytes (Schuh & Ellenberg 2007) and tracking of kinetochores at unprecedented resolution (Kitajima et al 2011). Powerful imaging techniques continue to propel new discoveries that could explain why female meiosis is error-prone (Holubcova et al 2015, Lister et al 2010, Nakagawa & FitzHarris 2017, Zielinska et al 2015) as well as which meiosis-specific mechanisms might be in place to prevent aneuploidy in eggs (Mogessie & Schuh 2017). Furthermore, cytogenetics has revealed unconventional principles of chromosome segregation (Ottolini et al 2015). A clearly emerging picture is that meiosis in mammalian oocytes has even more unique features than previously thought.

Despite these important advances, fundamental questions remain to be answered. The participation of other acentriolar spindle assembly mechanisms such as the Augmin pathway (Goshima & Kimura 2010) is yet to be substantiated in mammalian oocytes. Furthermore, our understanding of how the spindle is assembled in oocytes of non-rodent mammals, including humans, is still very limited. Understanding how spindle microtubules and kinetochore-fibers are organized at the structural level may also provide further insights into the error-prone nature of meiosis in oocytes. Indeed, combining live cell imaging with thorough three-dimensional reconstruction of microtubules from electron

tomograms has recently revealed unexpected kinetochore-fiber organization in mitotic spindles of early *C. elegans* embryos (Redemann et al 2017). That actin promotes faithful chromosome segregation in mouse oocytes also begs the question whether this function is conserved in oocytes of other mammalian species. This unexpected finding also warrants revisiting another long-standing question that transcends meiosis: does actin perhaps also play a role in mitotic chromosome segregation in certain cell types (Sandquist et al 2011)?

The finding that sister-kinetochores are predominantly split in human oocytes opens a new avenue for mechanistic studies of aneuploidy in mammalian eggs. How does the meiotic spindle cope with rotated and twisted bivalents during chromosome segregation?

Finally, understanding the molecular details of meiosis in mammalian oocytes demands efficient and acute protein depletion tools. RNA interference methods suitable for depletion of proteins at early stages of oocyte development are now established (Pfender et al 2015). However, a few problems still prevail: some proteins are stored from even earlier developmental stages while others are required for oocyte development, thus precluding their functional characterization in meiosis. Innovation of acute protein depletion or inhibition methods such as Trim-away (Clift *et al* 2018, in press) should overcome these challenges. In doing so, it will allow us to effectively address those key questions whose answers constitute a major step forward in our understanding of meiosis in mammalian oocytes.

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FIGURE LEGENDS

Figure 1. Stages from oocyte growth to formation of the early mouse embryo. During growth of oocytes in ovarian follicles, homologous chromosomes pair and initiate recombination. A glycoprotein shell called zona pellucida (shown in yellow) surrounds and protects the oocytes and embryo until implantation. Before meiotic resumption, the nucleus relocates to the oocyte center. Once every menstrual cycle a cohort of fully grown oocytes is released from prophase I arrest and undergoes two rounds of asymmetric divisions. In meiosis I, the spindle assembles at the center and migrates to the oocyte surface where half of the chromosomes are eliminated into the first polar body. The remaining half of the chromosomes is captured by and organized on the second meiotic spindle that is assembled in the egg, which is now arrested in metaphase II. The second meiotic spindle assembles around the remaining half of chromosomes beneath the surface of the egg, which arrests in metaphase II. Fertilization triggers the segregation of half of the sister chromatids into the second polar body. Finally, the haploid pronuclei form and migrate to unite at the zygote center where the first mitotic spindle of the embryo is assembled before symmetric division.

Figure 2. Acentriolar meiotic spindle assembly in mouse oocytes. Upon meiotic resumption, aMTOCs start to nucleate microtubules asters and converge towards the nucleus where they are stretched and fragmented in a dynein-dependent manner. After

nuclear envelope breakdown (NEBD), a microtubule ball forms with aMTOCs in its interior and the chromosomes are distributed in a circular fashion forming a distinctive prometaphase belt. The aMTOCs are then expelled outwards and the microtubule ball bipolarizes by the action of the KIF11 motor. Bipolar spindle formation is completed when aMTOCs are coalesced and spindle poles are stabilized, a process that involves the microtubule-associated proteins NuMA and HURP.

Figure 3. Chromosome segregation by meiotic spindles in mouse oocytes. Microtubules drive the positioning of chromosomes within the spindle. In mouse oocytes, spindle actin helps to bundle microtubules into functional k-fibers. This promotes accurate alignment and segregation of chromosomes. Loss of spindle actin reduces k-fiber stability and leads to chromosome misalignment in metaphase and lagging chromosomes at anaphase.

Figure 4. Error-prone chromosome segregation by meiotic spindles in human oocytes. Sister-kinetochores in human oocytes become increasingly separated with maternal age. Kinetochore splitting promotes incorrect microtubule attachments and unconventional geometries of bivalents. A single kinetochore can be attached to k-fibers originating from both spindle poles (merotelic attachment, red k-fibers), which is a leading cause of lagging chromosomes at anaphase (1). Some bivalents in old human oocytes are prematurely separated into univalents (2) whereas others are fully rotated (3), which could both lead to premature separation of sister chromatids at anaphase I.

Figure 5. Nuclear positioning in mouse oocytes. Before it breaks down in meiosis I, the nucleus relocates to the center of the growing oocyte. A dynamic network of actin and vesicles is present in the cytoplasm of mouse oocytes. Vesicles close to the oocyte surface move rapidly (large arrows), while more central vesicles move more slowly (small arrows), thereby creating a gradient of vesicle motility. This gradient relies on Myosin-5b and generates propulsion forces that center the oocyte nucleus (red arrow, nuclear centration). In mature oocytes, the nucleus is maintained at the center (nuclear anchorage) through yet unknown mechanisms. Possibly, microtubules might counteract actin-dependent forces that could otherwise drive the nucleus towards the periphery.

Figure 6. Asymmetric spindle positioning in mouse oocytes. Spindle relocation to the oocyte surface relies on two actin motors, Myosin-5b and Myosin-2. Myosin-5b is required for network dynamics, and thus supports spindle migration. Alternatively, spindle relocation may be directly coupled to Myosin-5b-mediated outward-directed transport of the vesicle-actin network.

Maintenance of the meiosis II spindle at the oocyte cortex relies on recruitment and activation of the Arp2/3 complex by a Ran-GTP gradient that forms around the chromosomes. An actin flow generated by Arp2/3 may translate into cytoplasmic streaming that pushes the spindle towards the cortex.

Figure 1

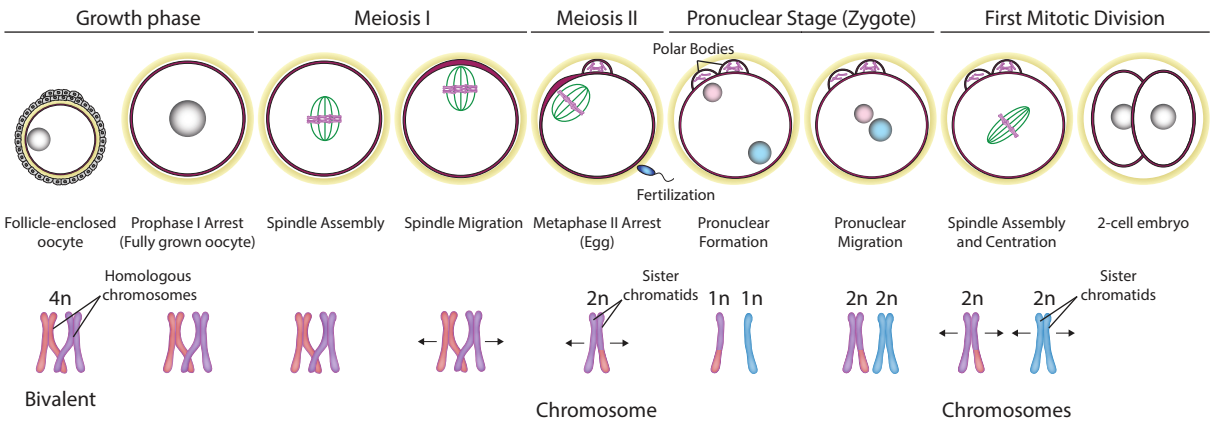

Growth phase
Meiosis I
Meiosis II
Pronuclear Stage (Zygote)
First Mitotic Division
Polar Bodies
Fertilization
Follicle-enclosed oocyte
Prophase I Arrest (Fully grown oocyte)
Spindle Assembly
Spindle Migration
Metaphase II Arrest (Egg)
Pronuclear Formation
Pronuclear Migration
Spindle Assembly and Centration
2-cell embryo
4n
Homologous chromosomes
2n
Sister chromatids
1n 1n
2n 2n
2n
2n
Sister chromatids
Bivalent
Chromosome
Chromosomes

Figure 2

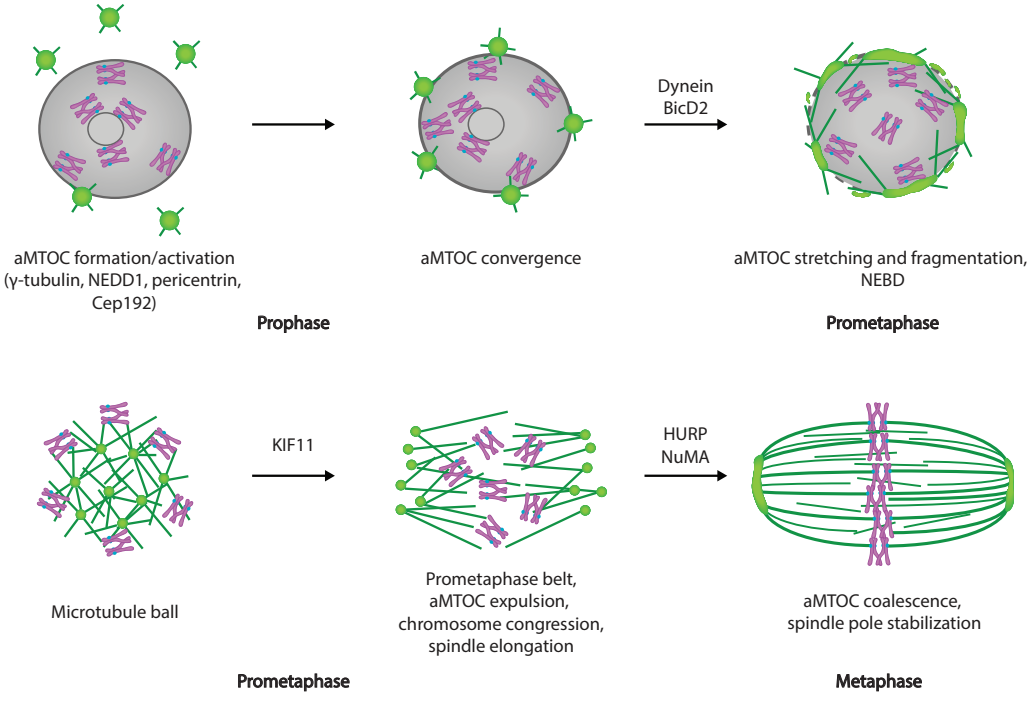
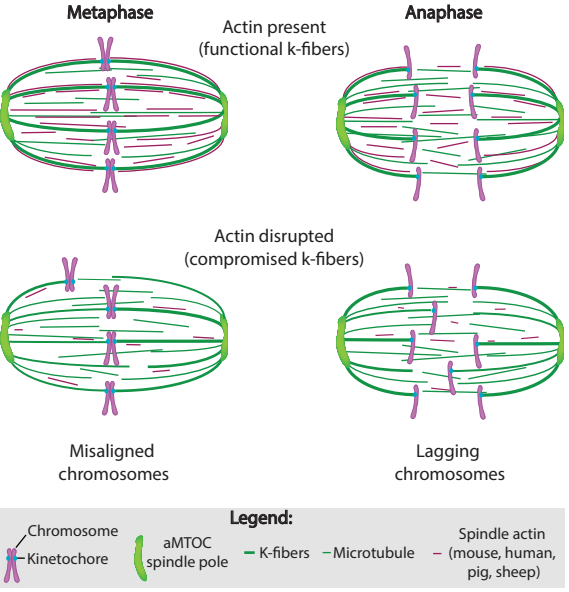

Dynein
BicD2
aMTOC formation/activation
(γ-tubulin, NEDD1, pericentrin,
Cep192)
aMTOC convergence
aMTOC stretching and fragmentation,
NEBD
Prophase
Prometaphase
KIF11
HURP
NuMA
Microtubule ball
Prometaphase belt,
aMTOC expulsion,
chromosome congression,
spindle elongation
aMTOC coalescence,
spindle pole stabilization
Prometaphase
Metaphase
Legend:
Chromosome
Kinetochore
Microtubule
aMTOC
Microtubule
Spindle pole

Figure 3


Metaphase
Anaphase
Actin present
(functional k-fibers)
Actin disrupted
(compromised k-fibers)
Misaligned
chromosomes
Lagging
chromosomes

Legend:
Chromosome
Kinetochore
aMTOC
spindle pole
K-fibers
Microtubule
Spindle actin
(mouse, human,
pig, sheep)

Figure 4

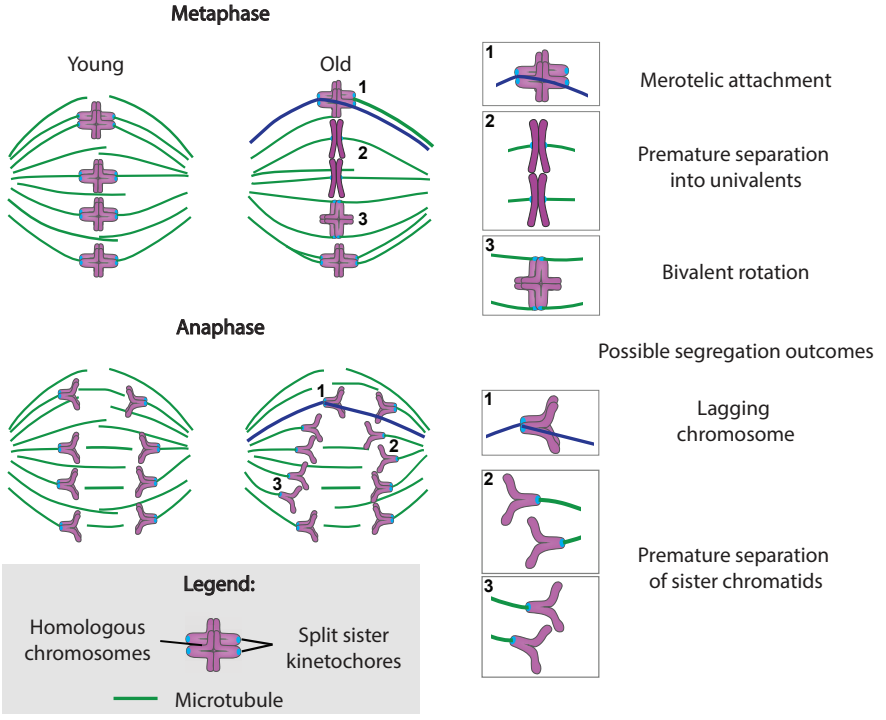

Metaphase
Young
Old
1
2
3
Anaphase
1
2
3
Legend:
Homologous chromosomes
Split sister kinetochores
Microtubule
1
Merotelic attachment
2
Premature separation into univalents
3
Bivalent rotation
Possible segregation outcomes
1
Lagging chromosome
2
3
Premature separation of sister chromatids

Figure 5

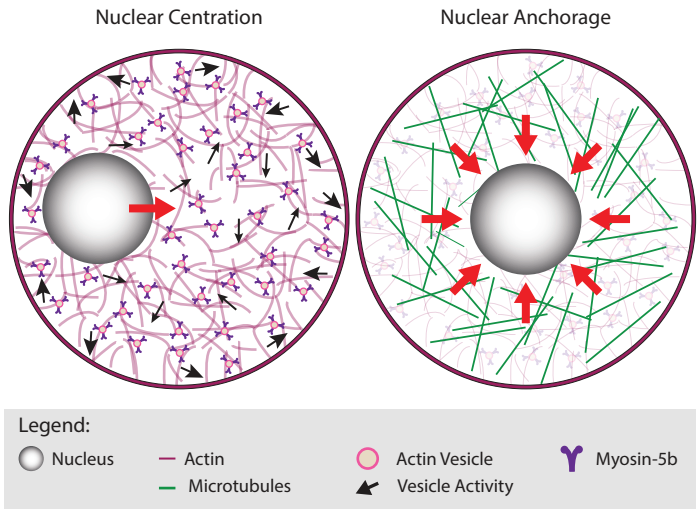
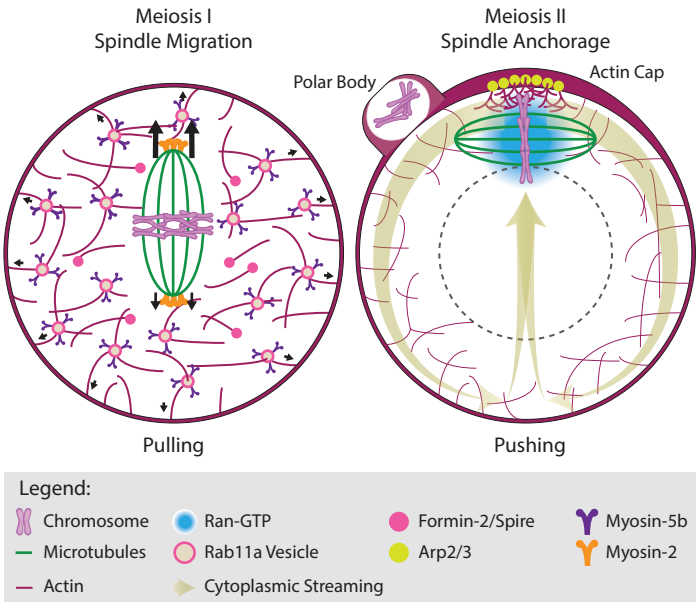

Nuclear Centration
Nuclear Anchorage
Legend:
Nucleus
Actin
Microtubules
Actin Vesicle
Vesicle Activity
Myosin-5b

Figure 6


Meiosis I
Spindle Migration
Meiosis II
Spindle Anchorage
Polar Body
Actin Cap
Pulling
Pushing
Legend:
Chromosome
Microtubules
Actin
Ran-GTP
Rab11a Vesicle
Cytoplasmic Streaming
Formin-2/Spire
Arp2/3
Myosin-5b
Myosin-2